# STAINING AND EVALUATION OF ROOTS FOR VESICULAR-ARBUSCULAR (V-A) MYCORRHIZAE

Many researchers have reported on minor modifications of the methods described by Phillips and Hayman (1970) for staining and evaluating vesicular-arbuscular (VA) mycorrhizae. The specific plant species: fungus species combination being utilized may influence the effectiveness of each procedure; refinements must be worked out by the individual researcher. One significant alteration has been to eliminate the use of phenol in any staining procedure.

## I. Staining Procedure

The following methods can be used effectively. Each method assumes that washed and sectioned roots (random or uniform lengths) are to be stained (see Gridline-intersect method; pg. 2).

### Trypan blue in lactic acid

| | |
|---|---|
| lactic acid | 100 mL |
| glycerol | 200 mL |
| deionized water | 200 mL |

(or any volume where the ratio of 1:2:2 [v/v] is maintained plus 0.3 g trypan blue per 500 mL volume (0.06%)

### A. No-heat Method

Place succulent (fine, feeder) roots in tissue cassettes. Submerge cassettes in ca. 10% (100 g KOH pellets/900 g $H_2O$) for approx. 12 hr. Pour off KOH, rinse cassettes in deionized water three times, and soak cassettes in the last change of water for at least 15 min. Acidify the last change of water by using approx. 5 mL 10% HCl per 250 mL deionized water. This is an absolute must as trypan blue staining is pH-sensitive.

- The cassettes are reusable, resistant to acids and bases, and can be marked permanently using a graphite lead pencil.
- For succulent roots, the stain can be reused at least 3 times before ineffective staining occurs. Filter with cheesecloth in between uses.
- Roots from woody plant species may require additional clearing of tannic acids, etc. E.g., following clearing in KOH, clear in 10% (3% a.i.) hydrogen peroxide for about 6 hr. Rinse three times and proceed as above.

B. Heat Method

This procedure is similar to the 'no-heat' procedure. Heat is applied at the clearing and staining steps for 60 min at 90° C (60 min in heated KOH, 60 min in heated stain). Boiling should not occur as this can displace root segments from the capsules, and can lead to the destruction of root cortical tissue.

- Alternative methods involve autoclaving roots in the KOH for 15 min (121 C, 15 psi), or heating the roots in KOH for up to one hour.

Regardless of the staining procedure some root segments will escape through casette openings. The amount (length, weight) of loss can be estimated and used to adjust total lengths in each cassette. For this reason, mycorrhizal and nonmycorrhizal samples should be kept separate where possible during the staining procedure.

## II. Evaluation of VA Mycorrhizae in Root Systems

This method (based on much earlier methods of Newman; Tennent; and Marsh) is from a report by Giovannetti & Mosse (1980; New Phytol. 84:489-500) in which four popular methods were compared. The meaningfulness of each method is subjective; however, the gridline-intersect method was the largest root sample weight and provides a maximum amount of data collected from each sample.

A. Gridline-intersect method

Stained root samples (prepared by randomly sampling root segments from each root system, accurately weighing to either 0.5 or 0.25 g fresh weight of root segments, and staining with one of the methods listed above) are distributed randomly across a petri dish that has been etched with lines (according to the cited paper). As little water as possible (approx. 10 mL) is added to the petri dish to distribute roots. A drop of dilute surfactant (household dish soap) is helpful in distributing roots in a thin layer of water. Using a dissecting microscope (20 - 30 X), each gridline is evaluated for the number of root-line intersects and/or the presence, type, or intensity of colonization (see Ambler and Young, 1977, Soil Sci. Soc. Amer. J. 41:551-556) at each intersect.

Data are summed over field of views and are expressed as:

$$(X/Y) \times 100 = Z\% \qquad \text{(Equation 1)}$$

where, X = number of mycorrhizal intersects, Y = total number of intersects, and Z = the % of total centimeters of root length infected. According to Giovannetti & Mosse (1980), a derivative equation:

$$R = \frac{A \pi N}{2H} \qquad \text{(Equation 2)}$$

also can be simplified to:

$$R = X, \text{ or} \qquad \text{(Equation 3)}$$

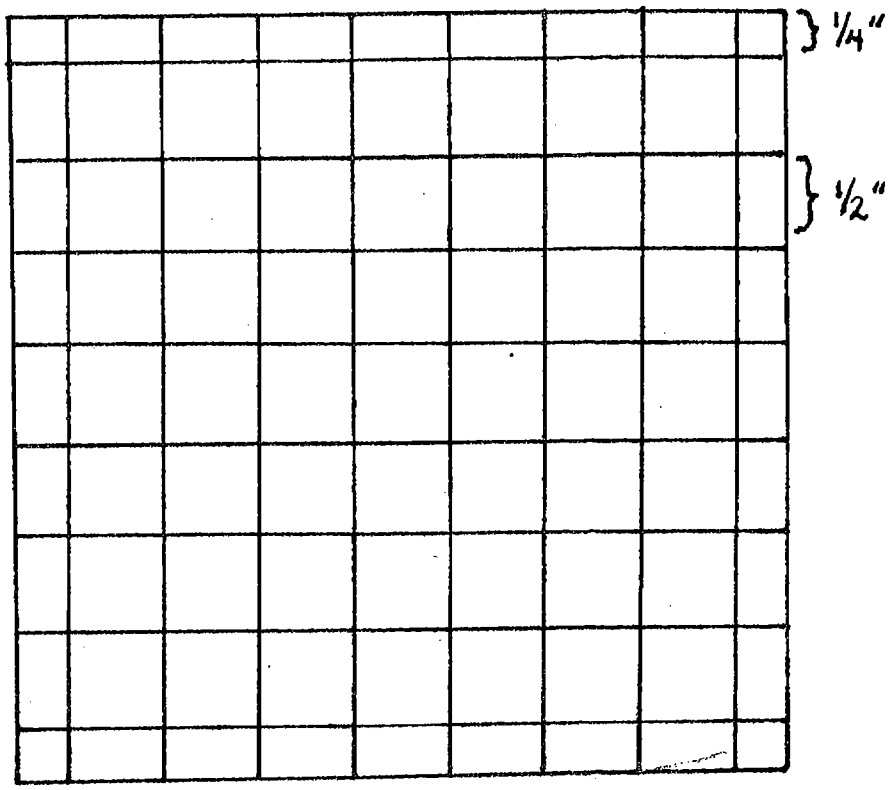
$$R = Y$$

where X is equal to the root length (in cm) colonized by mycorrhizal fungi, and Y is equal to the total root length (in cm) per fresh weight root subsample. As it turns out, only one-half of the total lines may be counted to estimate intersects accurately. Determine if this reduction in counting can be applied to your samples; i.e., fibrous roots may apply; however, coarse roots will not. One rule-of-thumb is, if there are $< 200$ intersects $0.25\ g^{-1}$ sample, you must count all root-line intersects.

References:


1. Giovannetti, M., and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.

2. Phillips, J.M., and Hayman, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55:158-161.




Grid-line intersect pattern - sensu Giovanneti & Mosse (1980) New Phytol. 84:489-500.

Place 10-cm-diameter petri dish upside down and adjacent to at least two corners: i.e.,

and score on the outside with probe.